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The Spectrum of Low-Density Lipoprotein Receptor Mutations in a Large Turkish Cohort of Patients with Familial Hypercholesterolemia

Ayberk Turkyilmaz, MD,[1] Erdal Kurnaz, MD,[2] Ceren Alavanda, MD,[3] Oguzhan Yarali, MD,[4] Emine Kartal Baykan, MD,[5] Dilek Yavuz, MD,[6] Atilla Cayir, MD,[7] and Pinar Ata, MD[3]

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Abstract

Background: Monogenic hypercholesterolemia with Mendelian inheritance is a heterogeneous group of diseases that are characterized by elevated plasma low-density lipoprotein cholesterol (LDL-C) levels, and the most common form of this disorder is autosomal-dominant familial hypercholesterolemia (FH).
Methods: A total of 104 index cases with the clinical diagnosis of FH were included in this study. Low-density lipoprotein receptor (*LDLR*) was sequenced using the Sanger sequencing method.
Results: Pathogenic/likely pathogenic variants were detected in *LDLR* in 55 of the 104 cases (mutation detection rate = 52.8%). Thirty different variants were detected in *LDLR*, three of which were novel. The total cholesterol and LDL-C values of the patients in the group of premature termination codon (PTC) mutation carriers were significantly higher than those of the patients in the group of non-PTC mutation carriers. A total of 87 patients (17 pediatric and 70 adult cases) were diagnosed with cascade genetic screening. Statin treatment was recommended to all 87 patients and was accepted and initiated in 70 of these patients.
Conclusions: This study is the largest patient cohort that evaluated FH cases in the Turkish population. Herein, we revealed the *LDLR* mutation spectrum for a Turkish population and compared the cases in the context of genotype–phenotype correlation. Genetic screening of individuals with suspected FH not only helps to establish their diagnosis, but also facilitates early diagnosis and treatment initiation in other family members through cascade screening.

Keywords: low-density lipoprotein receptor, genotype-to-phenotype, familial hypercholesterolemia, novel variants

Introduction

Monogenic hypercholesterolemia with Mendelian inheritance is a heterogeneous group of diseases that are characterized by elevated plasma low-density lipoprotein cholesterol (LDL-C) levels, and the most common form of this disorder is autosomal-dominant familial hypercholesterolemia (FH) (ADH). The incidence of this disease is ~1:300/1:500 in most populations.[1] However, on the contrary, autosomal recessive FH is extremely rare with an incidence of 1:160,000/1:250,000. FH is characterized by premature heart attack and stroke because of plasma LDL-C elevation and is associated with tendon xanthomata, xanthelasma, corneal arcus, and early atherosclerosis.[2]

In 70%–95% of all ADH cases, heterozygous pathogenic variants were located in low-density lipoprotein receptor (*LDLR*), *Apolipoprotein B* (*APOB*), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes involved in the clearance of LDL-C from the plasma. The *LDLR*, which is the most common genetic cause of ADH, is located on chromosome 19p13.2 and is ~45 kb in size and comprises 18 exons.[3]

[1]Department of Medical Genetics, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey.
[2]Department of Pediatric Endocrinology, Dr. Sami Ulus Obstetrics and Gynecology, Children's Health and Disease Training and Research Hospital, Ankara, Turkey.
[3]Department of Medical Genetics, Marmara University School of Medicine, Istanbul, Turkey.
Departments of [4]Medical Genetics and [5]Endocrinology, Erzurum City Hospital, Erzurum, Turkey.
[6]Department of Endocrinology, Marmara University School of Medicine, Istanbul, Turkey.
[7]Department of Pediatric Endocrinology, Erzurum City Hospital, Erzurum, Turkey.

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Mutations are divided into six classes depending on which protein domain is affected by the pathogenic variant in *LDLR*: class 1 mutations are receptor-negative mutations that form a null allele and cause a lack of receptor synthesis. They are associated with severe clinical course. Class 1 mutations often include nonsense, splice site, exonic deletions/duplications, and frameshift types. From classes 2 to 6, the type of mutations are missense and inframe indel, which are also called as receptor-defective mutations, and have been shown to be associated with a milder clinical course. Class 2 mutations cause defects in the transport of the LDLR molecule from the endoplasmic reticulum to the golgi, whereas class 3 mutations cause a defect in LDLR-ApoB binding, class 4 mutations cause an internalization defect, class 5 mutations cause a recycling defect, and class 6 mutations cause a defect in the transport of the LDLR molecule to the basolateral membrane.[4]

Heterozygous pathogenic variant in *APOB* is reported in 1%–5% of all ADH cases, and there are common variants that can be considered to be founder effects, particularly in certain populations. The p.Arg3527Gln variants are common in the European ancestry and p.Arg3527Trp variants in the Asian populations, respectively.[5] Less commonly, the gain-of-function variants in *PCSK9* that cause degradation of LDLR protein are associated with the ADH phenotype.[6]

Although FH is a common problem in the Turkish population, it frequently remains undiagnosed and therefore untreated. Most cases are diagnosed at the screening analysis by random measurements of the plasma cholesterol levels. It is therefore important to clarify the molecular etiology of these cases with suspected FH, in several aspects, as detailed hereunder: (i) The type of mutation is of utmost importance in terms of clinical follow-up and prognosis of the patient as well as in the treatment strategy and (ii) screening the relatives of the index cases with ''cascade genetic screening.'' After molecular diagnosis of the index cases, screening of asymptomatic family members could enable early diagnosis and access of the risk population in the treatment and prevention of cardiovascular complications.[7]

Herein, we aimed to determine the demographic and biochemical characteristics of FH as well as the incidence and distribution of pathogenic variants of *LDLR* in Turkish FH cases. Moreover, we compared the patients with and without pathogenic variants in terms of the plasma lipid levels, incidence of cardiovascular diseases, and physical examination results (tendon xanthoma, xanthelasma, and corneal arcus) and discussed the genotype–phenotype correlation.

Materials and Methods

Patients

In this study, 104 index patients (44 men and 60 women, mean age: 27.08 ± 17.20 years, age range: 2–81 years) with a clinical diagnosis of FH were included. Modified Simon Broome criteria or Dutch Lipid Clinic Network criteria were used for diagnosing FH in adult cases of age >18 years.[8–10] The diagnostic criteria for cases in the pediatric age group were as follows: (i) LDL-C level >190 mg/dL at two different timepoints after 3 months of diet, (ii) LDL-C level of the index case >160 mg/dL in the presence of premature coronary heart disease (CHD) in close relatives and/or hypercholesterolemia in the parents, and (iii) LDL-C level >130 mg/dL with genetic diagnosis in one of the parents, and (iv) exclusion of the secondary causes that may lead to hypercholesterolemia.[11,12]

Demographic characteristics, body mass index (BMI), serum lipid values [total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol, triglycerides levels], physical examination findings (*e.g.,* tendon xanthoma, xanthelasma, and corneal arcus), as well as the presence of a history of cerebrovascular disease (CVD) and premature CHD of the index cases were recorded. For evaluation of family history, the presence of hypercholesterolemia, premature CHD, peripheral vascular disease, and CVDs in the first- and second-degree relatives were evaluated. Secondary causes that may lead to hypercholesterolemia were excluded based on history, physical examination, and appropriate laboratory tests. The index cases were further evaluated with a detailed cardiovascular examination.

We obtained written informed consent for the study from the patients and/or their parents before their enrolment. This study was approved by the institutional ethics committee (Erzurum Training and Research Hospital, Approval No. 2020/16-168).

Molecular study

Genomic DNA was isolated from peripheral blood using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's recommendations. All exons of *LDLR*, exon–intron junctions, and the first 50 bases from each intronic region following the exon were amplified by polymerase chain reaction (PCR), as previously described.[13] Amplified PCR products were sequenced by the Sanger sequencing method using the ABI PRISM® 3130xl genetic analyzer (Applied Biosystems, Foster City, CA).

In silico analysis tools (DANN, DEOGEN2, EIGEN, MVP, Mutation Assessor, PrimateAI, REVEL, SIFT, PROVEAN, LRT, GERP, FATHMM-MKL, and Mutation Taster), the incidence of the variant in population databases (1000G, ExAC, and gnomAD), segregation analysis, and the American College of Medical Genetics and Genomics (ACMG) criteria were used to evaluate the pathogenicity of the novel variants detected.[14] The variants, which are defined as likely pathogenic and pathogenic in ClinVar and as ''disease mutation'' in HGMD® database, were found to be valuable to explain the patient's clinical presentation.

Statistical analyses

The Statistical Package for the Social Sciences (SPSS for Windows, Version 20.0, Chicago, IL) software was used for statistical analyses. The lipid values were adjusted for both sex and age in the index cases. Numerical variables were presented as mean and standard deviation, whereas categorical variables were presented as frequency and percentage values. The normality of the distribution was evaluated by the Kolmogorov–Smirnov test. Subsequently, binary group comparisons were conducted using the Mann–Whitney *U*-test. Categorical variables were compared using the chi-squared test. $P < 0.05$ was considered sufficient for statistical significance.

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Results

The mean age of the 104 index cases from 97 unrelated families (60 women and 44 men) was 27.08 ± 17.20 (age range: 2–81) years. Table 1 presents the demographic and clinical characteristics of the study cohort with the details of the sex distribution, mean standardized BMI and lipid values, prevalence of xanthoma, CHD, CVD, and peripheral artery disease (PAD) in mutation-positive ($n = 55$) and mutation-negative ($n = 49$) cases as well as total cases ($n = 104$). The prevalence of history of hyperlipidemia, CHD, CVD, and PAD in the first- and second-degree relatives of the index cases is also summarized.

Mutations were reported in 55 of the 104 index cases included in the study. The mean age of the mutation-negative cases (33.29 ± 16.25 years) was significantly higher than that of the mutation-positive cases (21.54 ± 16.22 years). The TC and LDL-C levels of mutation-positive cases (365.21 ± 110.79 and 292.94 ± 102.90 years, respectively) were significantly higher than those of mutation-negative cases (302.57 ± 33.88 and 223.57 ± 34.95 years, respectively). The incidence of xanthoma was reported to be significantly higher in mutation-positive cases than in mutation-negative cases. Higher incidence of hyperlipidemia, CHD, and PAD in the first- and second-degree relatives of mutation-positive cases were reported to be significant when compared with the family history of mutation-negative cases. We believed that the patients with LDL-C values >500 mg/dL in the pediatric patient group may have had biallelic *LDLR* mutation considering a first-cousin marriage between the parents. The serum lipid values of the parents of these patients were consistent with the ADH diagnostic criteria. In the genetic analysis, the pediatric index cases showed biallelic *LDLR* mutation, and their parents showed monoallelic *LDLR* mutation.

To examine the phenotypic effect of *LDLR* mutations, mutation carriers ($n = 55$) were divided into two groups as premature termination codon (PTC) mutation carriers and non-PTC mutation carriers. Among the PTC mutation carriers ($n = 17$), nonsense, splice site, and frameshift variants were detected that cause null alleles or premature truncations of LDLR. PTC mutations were associated with <2% normal receptor activity. In the non-PTC mutation carrier group ($n = 38$), missense and inframe variants causing residual receptor activity were detected. Non-PTC mutations were associated with 5%–60% of the normal receptor activity. When compared with the non-PTC mutation carrier group, the TC and LDL-C values in the PTC mutation carrier group (408.52 ± 102.38 and 335.05 ± 107.58, respectively) were significantly higher. Table 2 presents the demographic and clinical characteristics of the PTC mutation carrier and non-PTC mutation carrier cases.

Pathogenic/likely pathogenic variants were detected in *LDLR* in 55 of 104 cases (mutation detection rate = 52.8%). In *LDLR*, 30 different variants were detected, 20 of which were missense (66.6%), 2 were nonsense (6.6%), 2 were small insertions (6.6%), 2 were inframe deletions (6.6%), 2 were splice-site variants (6.6%), 1 was small deletion (3.3%), and 1 was synonymous (3.3%) variant (Fig. 1). The most common variations were c.1729T>C (p.W577R), c.1478_1479delCT (p.S493Cfs*42), and c.2389G>A (p.V797M). Table 3 lists the most common pathogenic variants and the phenotype of the patients carrying them. The variations detected in 61.8% ($n = 34$) of the cases were on the epidermal growth factor (EGF)-precursor homology domain. Three novel variants were detected, namely, c.457_465del (p.F153_C155del), c.1187-1delG, and c.2139A>G (p.T713T). Table 4 lists the ACMG, ClinVar, and HGMD classifications and the novelty status of all variations.

Table 1. Demographic and Clinical Characteristics of Mutation-Positive and Mutation-Negative Patients

	All FH patients (n = 104)	*Mutation-positive (n = 55)*	*Mutation-negative (n = 49)*	*Difference between genotype groups (P)*
Age, years	27.08 ± 17.20	21.54 ± 16.22	33.29 ± 16.25	**<0.001**
Mean age of pediatric cases (41.3%)	10.20 ± 4.20	10.05 ± 4.09	10.62 ± 4.69	—
Mean age of adult cases (58.7%)	38.62 ± 12.32	37.52 ± 12.79	39.86 ± 11.81	—
Female, %	57.69	54.55	61.22	1
Male, %	42.31	45.45	38.78	0.36
BMI SDS	–0.02 ± 0.76	–0.11 ± 0.84	0.08 ± 0.66	0.328
TC, mg/dL	335.70 ± 89.20	365.21 ± 110.79	302.57 ± 33.88	**<0.001**
LDL-C, mg/dL	260.25 ± 85.63	292.94 ± 102.90	223.57 ± 34.95	**<0.001**
HDL-C, mg/dL	61.75 ± 58.96	53.90 ± 14.15	70.56 ± 84.17	**0.01**
TG, mg/dL	96.63 ± 33.20	92.95 ± 29.31	100.77 ± 36.99	0.227
Prevalence of xanthoma, %	27.88	38.18	16.32	**0.016**
Prevalence of premature CHD, %	5.77	7.27	4.08	0.41
Prevalence of CVD, %	0	0	0	—
Prevalence of PAD, %	2.88	1.81	4.08	0.56
Prevalence of family history with hyperlipidemia, %	74	100	44.89	**<0.001**
Prevalence of family history with CHD, %	43.27	63.63	20.40	**<0.001**
Prevalence of family history with CVD, %	16.34	18.18	14.28	0.46
Prevalence of family history with PAD, %	12.5	20	4.08	**0.013**

Statistically significant changes are marked as bold.

Family history: First- and second-degree relatives.

BMI, body mass index; CHD, coronary heart disease; CVD, cerebrovascular disease; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PAD, peripheral artery disease; SDS, standard deviation score; TC, total cholesterol; TG, triglycerides.

Table 2. Demographic and Clinical Characteristics of Premature Termination Codon Mutation-Positive and Nonpremature Termination Codon Mutation-Positive Patients

	All mutation-positive patients (n = *55*)	*PTC mutation* (n = *17*)	*Non-PTC mutation* (n = *38*)	*Difference between genotype groups* (P)
Age, years	21.54 ± 16.22	21.76 ± 17.16	21.44 ± 16.02	0.8
Mean age of pediatric cases (58.1%)	10.05 ± 4.09	9.87 ± 3.43	10.36 ± 4.12	—
Mean age of adult cases (41.9%)	37.52 ± 12.79	39.24 ± 13.76	36.66 ± 12.86	—
Female, %	54.55	41.18	60.53	**0.003**
Male, %	45.45	58.82	39.47	0.317
BMI SDS	–0.11 ± 0.84	–0.1 ± 0.68	–0.12 ± 0.90	0.62
TC, mg/dL	365.21 ± 110.79	408.52 ± 102.38	345.84 ± 110.16	**<0.001**
LDL-C, mg/dL	292.94 ± 102.90	335.05 ± 107.58	274.10 ± 96.28	**<0.001**
HDL-C, mg/dL	53.90 ± 14.15	53.47 ± 13.75	54.09 ± 14.50	0.45
TG, mg/dL	92.95 ± 29.31	99.88 ± 36.12	89.85 ± 25.64	0.42
Prevalence of xanthoma, %	38.18	35.29	39.10	0.06
Prevalence of premature CHD, %	7.27	5.88	7.89	0.317
Prevalence of family history with CHD, %	63.63	82.35	55.26	0.237
Prevalence of family history with CVD, %	18.18	17.46	18.42	0.206
Prevalence of family history with PAD, %	20	5.88	26.31	0.07

Statistically significant changes are marked as bold.
Family history: First- and second-degree relatives.
PTC, premature termination codon.

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Parents and siblings of 55 mutation-positive cases were investigated by cascade genetic screening. Mutations were detected in 1 parent (32 patients) of all 32 pediatric cases and in 30% of their siblings (15 patients). Mutations were also detected in 1 of the parents of 20 of the 23 adult cases and in 35% of their siblings (20 patients). The average age of the adult cases with mutation detected by cascade genetic screening was 30.12 ± 12.12 (range: 18–43) years. The average age of pediatric cases with mutations detected by cascade genetic screening was 11.07 ± 5.45 (range: 5–17) years. Lifestyle changes (including diet, smoking cessation, and exercise) were recommended to all 55 index cases and 87 of their relatives with mutations. Statin treatment was recommended to 93 adult patients with mutations; of these, 80 patients accepted the treatment and the treatment was initiated (mean age of treatment initiation: 32.23 ± 11.43 years). Patients <8 years of age were followed up with diet therapy alone. Statin treatment was recommended to 39 pediatric patients with mutations, the parents of 32 patients accepted the treatment and treatment was accordingly initiated (mean age of treatment initiation: 12.05 ± 3.24 years).

Discussion

In our study, *LDLR* sequence analysis was performed in 104 Turkish patients diagnosed with ADH, and 52.8% of our cases had mutations. To the best of our knowledge, this study was the largest study conducted in the Turkish population. Using the ''cascade genetic screening'' approach, family members of our mutation-positive cases were tested, and we could diagnose and treat the asymptomatic cases in the early period. When compared with previous studies on Turkish population, sequence analysis of all coding regions and intronic regions closer to the exons of *LDLR* revealed rare genetic variations in our population.[15–20] A total of 30 different variations were detected; in nearly 50% of all

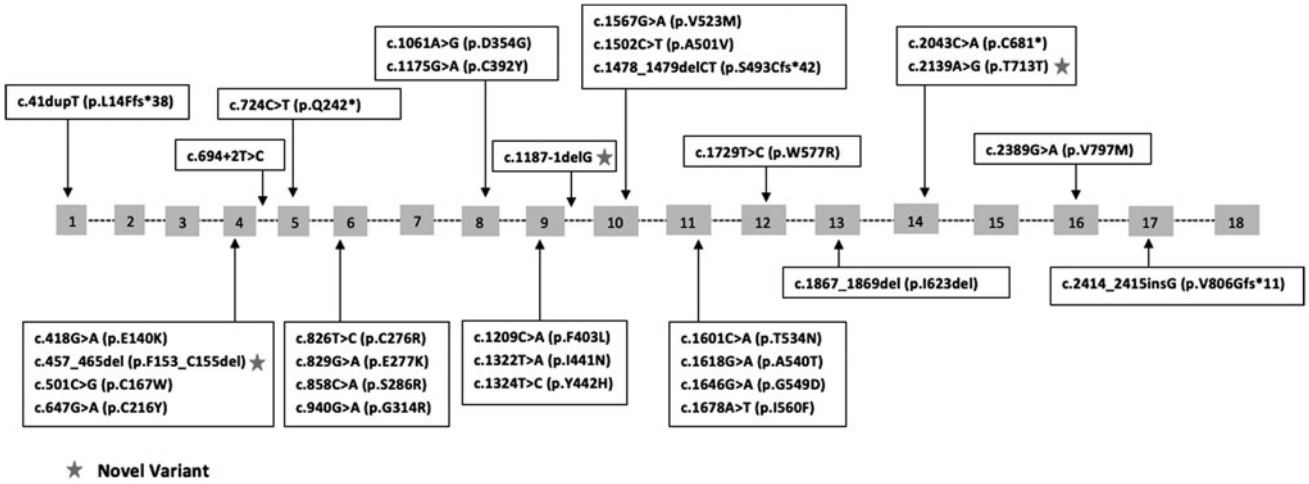


FIG. 1. The spectrum of the *LDLR* gene mutations in this study. All *boxes* indicate exons of the *LDLR* gene and *dashed lines* indicate introns of the gene. The novel variants in this study were shown with an *asterisk*. *LDLR*, low-density lipoprotein receptor.

Table 3. Most Common Variants and Their Clinical Characteristics in This Cohort

Variation	*No. of patients*	*TC, mg/dL*	*LDL-C, mg/dL*	*HDL-C, mg/dL*	*TG, mg/dL*	*Prevalence of xanthoma, %*	*Prevalence of CHD, %*
c.1729T>C (p.W577R)	9	332.66 ± 48.12	264.33 ± 40.49	50.37 ± 8.29	89.77 ± 16.96	55.5	11.1
c.1478_1479delCT (p.S493Cfs*42)	6	384 ± 39.02	310.16 ± 36.99	54.8 ± 6.71	95.16 ± 22.36	33.3	16.6
c.2389G>A (p.V797M)	4	354.5 ± 26.71	288.25 ± 21.51	48.05 ± 6.78	91 ± 18.83	75	—

these cases, the 6 most common mutations (p.W577R, p.S493Cfs*42, p.V797M, p.F153_C155del, p.I560F, and p.D354G) were responsible for genetic etiology. Variation c.1729T>C (p.W577R) was detected in 16.3% of the mutation-positive cases. Similar to previous studies concerning Turkish population, this was the most common variation. Functional studies demonstrated that this variation reduces the LDL receptor activity to <5% of the wild-type receptor's activity.[21] When the frequencies of mutations in the populations of Asia, Balkans, Cyprus, and Greece that are geographically close to Turkey were examined, this variation was not observed in the neighborhood populations, except in an Iranian population.[22–24] Accordingly, this variation could be considered as a founder mutation of the Turkish population.

Moreover, it was found that 3 of 30 different variations were novel and not reported previously in the literature. The novel c.457_465del (p.F153_C155del) inframe deletion, located within exon 4 of *LDLR*, was detected in a 12-year-old boy with hypercholesterolemia. It was also detected that his father and both the siblings had a history of hypercholesterolemia; at the segregation analysis, it was noted that other cases with similar phenotypes also carried mutations. Thus, it has been predicted that this variation, which is considered to be likely pathogenic according to the ACMG criteria, may affect the functioning of the EGF-precursor homology domain and impair the receptor transport or ligand binding.[14] However, this prediction requires further investigation and confirmation with functional studies.

Novel c.1187-1delG variation was detected in the splice acceptor region in intron 9 of *LDLR*. It was predicted that this variation, which is considered to be pathogenic according to the ACMG criteria, may disrupt splicing in *in silico* analyses.[14] The effect of this variation on splicing should be investigated by studies at the RNA and protein levels.

Table 4. Detected Low-Density Lipoprotein Receptor Gene Variants in This Cohort

Gene location	*Nucleotide change*	*Amino acid change*	*ACMG classification*	*HGMD/ClinVar*	*Novelty*
Exon 1	c.41dupT	p.L14Ffs*38	Pathogenic	DM/P	PR
Exon 4	c.418G>A	p.E140K	Pathogenic	DM/P	PR
Exon 4	c.457_465del	p.F153_C155del	Likely pathogenic	—	**Novel**
Exon 4	c.501C>G	p.C167W	Pathogenic	DM/P	PR
Exon 4	c.647G>A	p.C216Y	Pathogenic	DM/LP	PR
Intron 4	c.694 + 2T>C		Pathogenic	DM/P	PR
Exon 5	c.724C>T	p.Q242*	Pathogenic	DM/P	PR
Exon 6	c.826T>C	p.C276R	Pathogenic	DM/LP	PR
Exon 6	c.829G>A	p.E277K	Likely pathogenic	DM?/CIP	PR
Exon 6	c.858C>A	p.S286R	Pathogenic	DM/LP	PR
Exon 6	c.940G>A	p.G314R	Pathogenic	DM/VUS	PR
Exon 8	c.1061A>G	p.D354G	Pathogenic	DM/P	PR
Exon 8	c.1175G>A	p.C392Y	Likely pathogenic	—/P	PR
Exon 9	c.1209C>A	p.F403L	Pathogenic	—/P	PR
Exon 9	c.1322T>A	p.I441N	Pathogenic	DM/LP	PR
Exon 9	c.1324T>C	p.Y442H	Pathogenic	DM/LP	PR
Intron 9	c.1187-1delG		Pathogenic	—	**Novel**
Exon 10	c.1478_1479delCT	p.S493Cfs*42	Pathogenic	DM/P	PR
Exon 10	c.1502C>T	p.A501V	Pathogenic	DM/P	PR
Exon 10	c.1567G>A	p.V523M	Pathogenic	DM/P	PR
Exon 11	c.1601C>A	p.T534N	Likely pathogenic	DM/LP	PR
Exon 11	c.1618G>A	p.A540T	Pathogenic	DM/P	PR
Exon 11	c.1646G>A	p.G549D	Pathogenic	DM/P	PR
Exon 11	c.1678A>T	p.I560F	Pathogenic	–/P	PR
Exon 12	c.1729T>C	p.W577R	Pathogenic	DM/LP	PR
Exon 13	c.1871_1873del	p.I624del	Pathogenic	DM/P	PR
Exon 14	c.2043C>A	p.C681*	Pathogenic	DM/P	PR
Exon 14	c.2139A>G	p.T713T	VUS	—	**Novel**
Exon 16	c.2389G>A	p.V797M	Pathogenic	DM/P	PR
Exon 17	c.2416dupG	p.V806Gfs*11	Pathogenic	DM/P	PR

Novel variants are marked as bold.

ACMG, American College of Medical Genetics and Genomics; CIP, conflicting interpretations of pathogenicity; DM, disease mutation; HGMD, Human Gene Mutation Database; LP, likely pathogenic; P, pathogenic; PR, previously reported; VUS, variant of uncertain significance.

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In a 6-year-old girl, novel c.2139A>G (p.T713T) synonymous variant was detected within exon 14 of *LDLR* in a homozygous manner. The serum TC and LDL-C values of the patients were 781 and 735 mg/dL, respectively, and her parents' values were consistent with the clinical presentations of ADH. It has been predicted that c.2139A>G (p.T713T) synonymous variation may disrupt splicing, inducing a change in the sequence of consensus splice donor site. Mutation Taster® program evaluates this variation as disease causing and Human Splicing Finder® program reveals that it disrupts the splicing. Its CADD (Combined Annotation Dependent Depletion) score has been given as 21.7, which denotes it as potentially disease causing.[25–27] This variation has never been encountered before in population studies (1000G, ExAC, and gnomAD). In the segregation analysis, the mother, father, grandmother, uncles, and grandfather with the ADH phenotype heterozygously carried the variation. This variation was considered to explain the clinical presentation of our case because the segregation analysis was consistent, *in silico* analyses supported pathogenicity, and it was a rare variant. However, the effect of the variation on splicing should be investigated by functional studies. When all variations were examined, 7 of them were in class 1 mutation group and 23 were in classes 2–6 mutation groups. The effects of some of the missense variations in classes 2–6 groups on the LDL receptor activity has been demonstrated by functional studies.[21] At the long-term follow-up, the type of mutation of the subjects could help us predict the development of CHD.[1,2] Molecular diagnosis and the mutation type detected were pivotal in prognosis and tailoring the follow-up.

The demographic and clinical characteristics of mutation-positive ($n = 55$) and mutation-negative ($n = 49$) patients were examined; the family history of hyperlipidemia, CHD, and PAD were found to be significantly more common in cases with mutations than in the groups without mutation (Table 1). Furthermore, the mean age of the patients with mutations was significantly lower than the group without mutations (Table 1). This observation may be attributed to predictive testing of the cases earlier considering the positive family history. Xanthoma was detected in only 38.18%, whereas CHD was detected in 7.27% of all mutation-positive cases (Table 1). This value may be attributed to the younger mean age at diagnosis (21.54 ± 16.22 years). In particular, for the pediatric age group, the absence of physical examination findings such as xanthoma and corneal arcus and the borderline elevation in the lipid values may lead to overlooking of the diagnosis.[22] In case of a positive family history, this group of cases should be carefully evaluated and genetically analyzed for early diagnosis.

Patients with PTC mutations ($n = 17$) showed significantly higher TC and LDL-C levels when compared with those with non-PTC mutations ($n = 38$) (Table 2). Similar results were reported in studies conducted within different populations.[1,2,28] When cases with PTC mutations were compared with those with non-PTC mutations in terms of xanthoma, CHD prevalence, and family history, no significant difference was reported. This observation may be associated with a low mean age at diagnosis (21.54 ± 16.22 years). It is already known that patients with PTC mutations develop CHD, PAD, and CVD more commonly, especially in the long-term follow-up.[1,2,29] Thus, it is considered that long-term follow-up of the cases may be important at this time point. Moreover, hyperlipidemia is not the only risk factor for the development of CHD. Diet, physical exercise, and the presence of additional comorbidities (such as diabetes mellitus and chronic renal failure) also play a role in CHD pathogenesis.[1]

All cases analyzed in this study were selected in accordance with internationally accepted diagnostic criteria, and the molecular diagnosis rate of the study was 52.8%. In different population studies, the rates of molecular diagnoses varied in the range of 18.5%–82%.[1–3,22,29,30] This variation in the diagnostic rates may be attributed to the variability of patient selection criteria and the difference in the scope of the molecular analysis methods applied. The molecular etiology could not be determined in certain cases, although the selection was made as per the clinical diagnostic criteria. This may be attributed to several reasons. (i) There may be pathogenic variations in *APOB, PCSK9, APOE*, and other rare genes responsible for the ADH phenotype. (ii) The promoter region of *LDLR* has not been evaluated yet; however, some variants in this region are also known to cause the ADH phenotype. (iii) Heterozygous gross deletions/duplications in *LDLR* could not be evaluated. In this respect, further analyses of the patients are necessary using the multiplex ligation-dependent probe amplification method for the exclusion of possible large deletions and/or duplications.

Conclusions

This study included the largest patient cohort evaluating ADH cases in the Turkish population. Herein, the *LDLR* mutation spectrum for the Turkish population has been revealed, and the cases with PTC and non-PTC mutations were compared in the context of genotype–phenotype correlation. Screening for FH in the index cases is crucial for diagnosing FH not only in index cases but also in their relatives. A total of 87 cases (17 pediatric and 70 adult cases) were diagnosed by using cascade genetic screening. Statin treatment was recommended to all 87 patients and was accepted and initiated in 70 of these patients. Younger mean age at diagnosis of the cases with mutations and screening of other family members by using ''cascade genetic screening'' enabled most cases to be diagnosed and treated in the early period. Moreover, early treatment of patients is of vital importance in reducing CHD-related morbidity and mortality in the long-term follow-up analyses.

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Address correspondence to:
Ayberk Turkyilmaz, MD
Department of Medical Genetics
Faculty of Medicine
Karadeniz Technical University
Ortahisar
Trabzon 61080
Turkey

E-mail: ayberkturkyilmaz@gmail.com